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LIQUID FUEL AND CHEMICALS
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CELLULOSIC BIOMASS

Prepared for
MONTANA DEPARTMENT of NATURAL RESOURCES and CONSERVATION

LIQUID FUEL AND CHEMICALS FROM
RENEWABLE CELLULOSIC BIOMASS

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Simple and inexpensive techniques for pretreating Montana lignocellulosic biomass materials were investigated to improve technology for converting them to fermentation feedstocks for ethanol production. Using state of the art apparatus and procedures, optimal autohydrolysis and alcoholic delignification pretreatment techniques were established for wheat straw and then applied to barley straw, lodgepole pine and Douglas fir substrates.

High yields of xylose sugars from hemicellulose were achieved for all biomass materials, but lignin removal at optimal conditions was only moderate for wheat and barley straw and essentially zero for the woody biomass. The conditions found optimal for wheat straw seem reasonable for barley straw, but are clearly not optimal for the woods based upon lignin removal.

Although cellulose concentration in pretreated straw pulps exceeded 75% and lignin concentrations were roughly 60% below starting levels, acid hydrolysis rates for all pulps were low. Enzyme hydrolysis rates, although not tested, are also presumed low. Residual intractable lignin and unexpectedly high cellulose crystallinities in pulps are plausible reasons for low conversions of cellulose to glucose.

Ongoing research funded by MSU is continuing with emphasis upon more severe pretreatment conditions and delignification solvents other than aqueous ethanol.

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General Background

Since 1973 when OPEC decided to limit production and exports of petroleum and to control prices at elevated levels, there has been great incentive to develop alternative supplies of energy, especially alternative liquid fuels. Renewable biomass, especially biomass wastes and by-products of agriculture and the timber industry, is an attractive raw material for fuel ethanol production via fermentation. Wheat and barley straw and sawdust fall within this category of potential fuelstock raw material.

Montana has locally abundant supplies of straw and timber by-products throughout the state. Their relatively low costs compared with other fermentation substrates such as grain make these biomass materials especially interesting to fuels researchers. However, significant technical problems stand in the way of commercial use of these lignocellulosics for fermentation.

The cellulosic fraction of straw or woody biomass consists of hemicellulose and cellulose. Hemicellulose is a complex polymer whose monomer constituents are primarily pentose sugars (xylose). The structure of cellulose is well known, and for cellulose the monomer building block is the hexose sugar glucose. Technology exists today to ferment both glucose and pentose sugars to ethanol with excellent yields. The fundamental problem in using straw or woody biomass for fermentation involves production of these simple sugars from the parent cellulosic polymers.

Xylose can be obtained from hemicellulose in biomass by a simple aqueous cooking operation similar to that employed to hydrolyze starch to glucose. However, biomass usually contains at least twice as much cellulose as hemicellulose, and thus hydrolysis of cellulose to glucose becomes the main objective. Very little glucose is produced from cellulose under the cooking conditions that convert hemicellulose readily to xylose. High temperature (pressure) cooking for

longer intervals or use of strong acid to speed hydrolysis results in major glucose degradation.

Lignin, a complex phenolic high polymer, is the main culprit prohibiting rapid cellulose hydrolysis under mild conditions. Lignin is chemically very inert and largely insoluble in common solvents. This refractory behavior combined with the fact that lignin exists in the plant cell wall as a "sheath" around the cellulose blocks the access of hydrolytic agents to the cellulose. Thus, lignin removal or destruction is required to readily hydrolyze cellulose under mild conditions.

A second problem in cellulose hydrolysis seems to result from the morphology of the cellulose itself. Most cellulose exists in nature as highly crystalline material - the so-called alpha form. Even in the absence of lignin, crystalline cellulose hydrolyzes slowly due to the "tight" ordering of crystalline folds which again yield poor access for hydrolytic species. The less common amorphous (beta) form of cellulose hydrolyzes rapidly much as hemicellulose does when no lignin is present. Thus, rapid hydrolysis of cellulose without glucose degradation requires both elimination of at least some lignin and disruption of the cellulose crystals. Most current research into utilization of lignocellulose for fermentation is directed at overcoming these two problems.

Other Research

A bibliography of recent research into use of lignocellulosic biomass is given in the Appendix in Milestone Report #3. The content of individual references is generally given by the article or presentation title. Each of these references adds some perspective to our current work, but by far the most significant other research is that by Lora and Wayman (10,11) at the University of Toronto.

Lora and Wayman investigated high temperature, short duration aqueous cooking of woody biomass (poplar). They called this operation autohydrolysis since no hydrolytic species other than those existing or produced in the wood were employed. Many other researchers had previously investigated high temperature autohydrolysis of wood and straw, but not carefully over the shorter time frames studied by Lora and Wayman.

The interesting finding of the Toronto researchers was that an optimum autohydrolysis exposure time existed at every practical autohydrolysis temperature. This optimum point was determined by a subsequent delignification operation on autohydrolysis solid residues. The optimal autohydrolysis exposure time was reached when a maximum amount of lignin was removed by a strong solvent. As autohydrolysis temperature increased to near 200°C, optimal exposure time dropped to less than ten minutes.

The autohydrolysis and delignification work of Lora and Wayman was directed at pulping for paper manufacture and falls far short of having commercial significance. Autohydrolysis was conducted in small sealed bombs immersed in hot oil, thereby giving heat-up and cool-down periods significantly longer than the optimal exposure intervals. Also, lignin removal was accomplished with the solvent dioxane, a costly and highly toxic material unsuitable for commercial use, especially in a pretreatment for fermentation.

Project Strategy

The concept for the current project grows out of the Lora and Wayman research. Perhaps two simple pretreatment techniques, autohydrolysis and delignification with an inexpensive, nontoxic solvent, could be successfully combined to pretreat lignocellulose. Neither step by itself has previously been successful; autohydrolysis has generally been used at excessive, nonoptimal times of exposure and no inexpensive, nontoxic solvent is known for delignification of non-autohydrolyzed biomass.

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The choice of delignification solvent to be tested is somewhat arbitrary, but aqueous ethanol stands out as a prospect. Ethanol has only slight ability to remove lignin from untreated biomass, but the situation with optimally autohydrolyzed biomass may be much different. The Toronto research showed that lignin removal rates varied by a factor of five or more using dioxane at autohydrolysis times ranging from the optimum value to departures of $\pm$ 20 minutes. Since the autohydrolyzed residue would be wet anyway and since water poses no threat to fermentation, a choice of 50/50 water/ethanol by volume was made. Another advantage for this solvent is the fact that in any integrated pretreatment and fermentation process, aqueous ethanol would be available on site.

A requirement in the present work is the capability to determine the optimal autohydrolysis time quite precisely. A standard unstirred bomb autoclave as employed by Lora and Wayman is clearly unacceptable. A stirred autoclave having the capability for rapid heat-up and cool-down is indicated. Any commercial process based upon the present work would use continuous autohydrolysis with residence time dictated by the optimal exposure determined in the batchwise experimental apparatus.

This research project seeks to develop new technology that may ultimately yield an economical and technically practical route to fuel grade ethanol from lignocellulosic residues abundant in Montana. Specific technical objectives of the research are summarized below.

1) Develop optimal autohydrolysis and alcoholic delignification pretreatment techniques that give high yields of xylose sugars from hemicellulose and efficient removal of lignin from wheat straw. Test these techniques on barley straw, lodgepole pine and Douglas fir.

2) Consistent with the preceding objective, develop optimal autohydrolysis and delignification techniques that yield wheat straw pulp having significantly improved rate of cellulose hydrolysis to glucose for fermentation. Test pulps produced from barley straw, lodgepole pine and Douglas fir.

3) Develop state-of-the-art batchwise experimental apparatus and procedures to accomplish the research objectives.

4) Develop an overall enhanced capability to perform lignocellulosic biomass energy conversions within the Chemical Engineering Department at MSU. This capability would provide the base for further funding of energy research on Montana-specific biomass materials by the Engineering Experiment Station and others.

Equipment

The experimental autoclave used for autohydrolysis and delignification is described in detail in the Appendix in Milestone Report #1. A vendor drawing of the unit is also attached.

The Autoclave Engineers Model ZC100 has several unique features significant to this work. First, the magnetic coupling of the agitator shaft and the drive motor make it possible to agitate the vessel at pressures up to 2000 psig without leakage around the shaft. The vessel also has extra head ports to allow connection to a standard Parr bomb autoclave used as a steam generator/heater and to cold traps through which the vessel contents can be vented. Finally, the unit is sealed with the novel AE Zipper seal which allows rapid opening and closing of the vessel during complex experiments.

Sample substrates to be treated in the autoclave were contained in miniature "baskets" constructed from 200 mesh 316 stainless wire cloth. Eight baskets were attached at equal spacing around the bottom of the agitator shaft to form a paddle-like arrangement. Baskets were sealed with mechanically pinched closures and held about one gram of sample each. Thus, the agitator paddles contained the samples to be exposed to the autohydrolysis water.

The ZC100 autoclave is fitted with a 1000 watt external band heater, but early autohydrolysis work confirmed that heating by this mechanism alone was much too slow, and heat-up time between $150^{\circ}C$ - $200^{\circ}C$ would exceed anticipated optimal times-at-temperature. To speed up heating the AC100 autoclave was connected via stainless tubing to a 500 ml Parr bomb autoclave. The Parr bomb contained water at $300^{\circ}C$ from which steam could be vented to the ZC100 autoclave for rapid heating. Steam flow control was achieved with an AE stainless needle valve.

A diverse variety of glassware, analytical devices and reagent chemicals was used to analyze starting materials and

product residues in this research. Individual items are too numerous to discuss here, but full descriptions are given in the standard analysis methods. These methods are:

Ash Determination	ASTM D-1102
Gum/Resin Extraction	TAPPI T12 os-75
Lignin Determination	TAPPI T9 m
Cellulose Components	TAPPI T203 os-74

Major analytical units included a Soxhlet extractor, a chlorine diffusion cell, a constant temperature water bath and a potentiometric titrimeter. The hydrolysis experiments were conducted in simple laboratory glassware.

Two major equipment problems arose during the work and each caused significant delay. On start-up of the ZC100 autoclave, a persistent leak in the head of the unit was encountered. Attempts in our lab to find and seal the leak failed over a period of several weeks. Finally, the vessel manufacturer suggested changing an internal head locking seal. This change solved the problem, with careful examination revealing that the original o-ring seal in the locking mechanism was defective.

A second problem arose when a rupture disc in the head of the Parr bomb blew out during heat-up. No personnel were in the laboratory when the disc ruptured, perhaps averting an injury. Our estimate of Parr bomb pressure when the disc blew out was 1500 psig, and the disc was rated at 4000 psig. An investigation failed to reveal the cause of the disc failure. Our best guesses are that the disc was fatigued due to age and repeated heatings or was originally defective. To avoid any repeat of this dangerous incident, carefully inspected new discs were used and the autoclaves were repiped into a safer, more stable configuration.

<u>Materials</u>

Wheat straw used in this research was Pondera spring variety and barley was Clark's variety. Each straw was obtained from the Larry Van Dyke ranch in Amsterdam, Montana. Straw was baled in the fall of 1982 and was stored

in the field for five months and in our laboratory thereafter.

Lodgepole pine and Douglas fir sawdust samples were obtained from the Brand S sawmill in Livingston, Montana. Sawdust was obtained in the summer of 1984 from recently harvested green and dead timber. Precise characterization of these sawdust samples in terms of age and origin is impossible beyond the description given here.

Raw material samples were ground to 35-60 mesh in a Wiley hammer mill and stored in sealed containers prior to use.

All chemicals used in this work were reagent grade or purer. Distilled water was used throughout the work.

Procedures

Autohydrolysis was carried out in the ZC100 autoclave. Roughly eight grams of ethanol/benzene-extracted substrate was loaded into the agitator baskets along with 600 ml of water. The unit was sealed and heat applied via the band heater. During heat-up to 170°C the unit was vented several times to eliminate air. Agitation was fixed at 200 rpm. Fixed agitation at 200 rpm is discussed in Milestone Report #5 in the Appendix. The literature indicated no appreciable autohydrolysis of wood below 170°C. When 170°C was reached, auxiliary steam was throttled into the unit, raising temperature to the desired level within 90 seconds. Temperature control at the desired level was manually achieved by feathering the band heater and ZC100 vent valve.

When the desired exposure time was reached, the vessel was vented through an ice-cooled stainless steel cold trap until the temperature again reached 170°C, generally taking less than one minute. Further cooling was by ambient natural convection. Solid residue in several baskets was quantitatively removed for analysis with the rest left for subsequent delignification.

Aqueous alcoholic delignification was also conducted in the ZC100 as indicated. A liquid charge of 300 ml 95% ethanol and 300 ml distilled water was used to delignify autohydrolysis residues. Contact time at 150°C and 200 rpm agitation was one hour. The fixed delignification temperature of 150°C and 200 rpm agitation is discussed in Milestone Report #5 in the Appendix. Heating was accomplished using only the band heater and cooling as in autohydrolysis. Delignified solid residue was quantitatively removed from the baskets and then sequentially washed three times with fresh delignification liquor and two times with distilled water. Solid residue was then dried and stored for analysis.

Acid hydrolysis was conducted in standard glassware to determine delignified pulp quality from the standpoint of hydrolytic activity. A mild version of ASTM D-1106-56 was adopted after much experimentation. Two steps are involved. First dried straw or wood is hydrolyzed with 16 N sulfuric acid for one hour at 28°C in an Erlenmeyer flask immersed in a constant temperature water bath. An acid-to-pulp ratio of 100:1 is used. Next the acid is diluted to 3 N and hydrolysis continues for two hours at 80°C. This latter step is more of a wash than a hydrolysis. The hydrolyzed residue is then washed with water and dried for weight loss determination.

Further specific details of autohydrolysis, delignification and acid hydrolysis are given in the Appendix in Milestone Report #5. When any discrepancy exists between any milestone report and the statements or results in this final report, the latter is correct. Many revisions in methods, both experimental and calculations, evolved over the course of the work, rendering some information reported in milestone documents obsolete.

Analytical procedures used throughout this work were either ASTM or TAPPI standards whenever practical. These standard methods are lengthy and complex and will not be detailed here. Procedures are summarized in the Appendix in Milestone Report #2. Sample calculations are also given.

One serious problem developed in using TAPPI T203 os-74 for amorphous (beta) cellulose. Colorimetric titration endpoints developed for wood in the standard failed when the substrate was straw. We were forced to use potentiometric titration, making estimates of beta cellulose uncertain at best. However, since beta cellulose levels were always low in relation to crystalline (alpha) levels, this problem has little overall impact on the research.

A few final notes should be made regarding these standard analytical procedures. Since the chemistry of the species involved is complex beyond present understanding, it must be recognized that the methods somewhat arbitrarily define the substance under analysis. This definition probably only roughly approximates the actual concentration of the species in the natural substrate. Beyond this problem, the standards were developed for specific woods, not those used in this work and certainly not straw. Thus, further serious error may be involved in any given analytical determination. However, these historic methods are the only practical tools available to the biomass researcher and thus are widely used. Where critical analytical precision is needed, specific methods must be developed. Such development is rare in biomass research and is certainly well beyond the scope of this project.

Autohydrolysis/Delignification

In this work, determination of optimal autohydrolysis conditions is based upon a single substrate, wheat straw. The evaluations of autohydrolysis/delignification characteristics of barley straw, lodgepole pine and Douglas fir were performed at conditions found optimal for wheat straw, thus avoiding four wide-ranging investigations. The latter three substrates probably exhibit optimal behavior at conditions somewhat different from those for wheat straw, but such differences, if they exist, will not be determined in this project.

Optimal autohydrolysis is achieved at conditions that yield near complete hydrolysis and removal of hemicellulose and maximum lignin removal in the combined autohydrolysis/delignification operations. The cellulose hydrolysis characteristics of wheat pulps produced at optimal conditions is established separately by the acid hydrolysis method.

Autohydrolysis experiments were conducted at three temperatures ($175^{\circ}C$, $195^{\circ}C$, and $205^{\circ}C$) and for exposure intervals of 0-120 minutes. Exposure intervals are times-at-temperature and would correspond to residence times in continuous processing equipment.

During autohydrolysis the solid substrate loses significant weight as hemicellulose and, to a lesser extent lignin and cellulose are lost to the autohydrolysis liquor (water). Beyond this loss there is further weight loss in the alcoholic delignification step as lignin and to a much lesser extent hemicellulose and cellulose are lost.

Weight losses versus autohydrolysis time and temperature are given in Figures 1 and 2. Figure 1 is for the autohydrolysis step only, and Figure 2 gives total weight loss through both autohydrolysis and delignification operations. It is seen that weight losses in excess of 40% are encountered even at $175^{\circ}C$ when autohydrolysis exposure time exceeds one hour. As discussed in the Experimental section, delignification was conducted at fixed conditions:
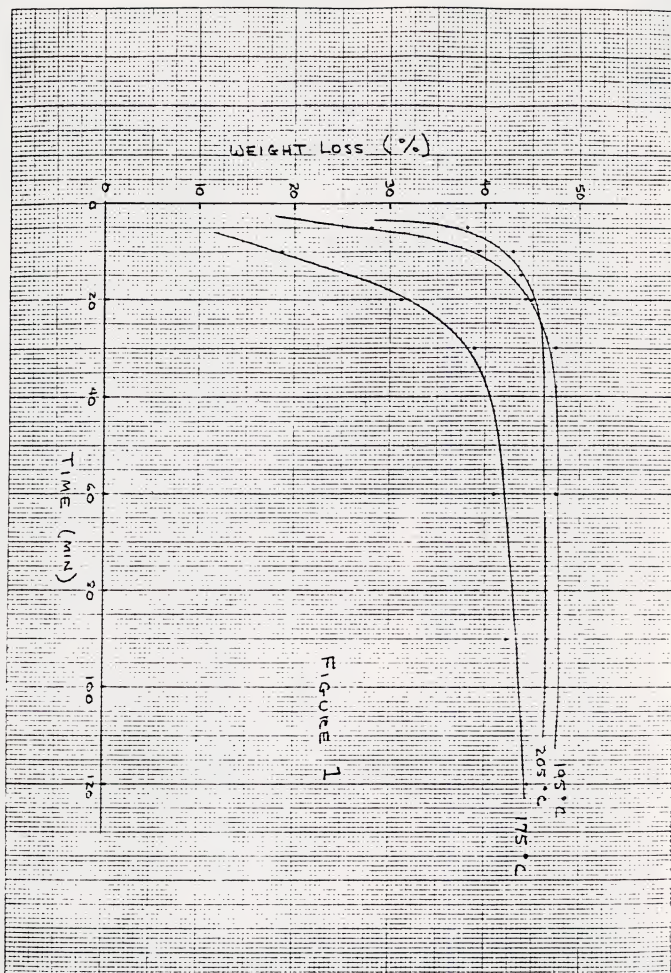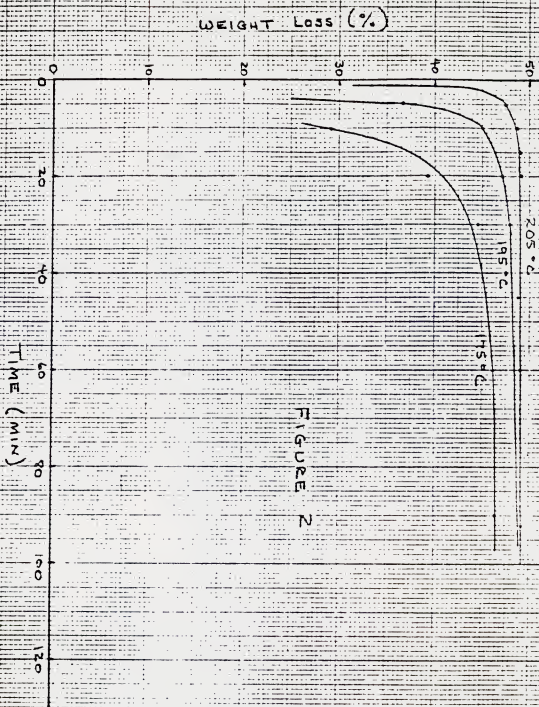
14

FIGURE 1

FIGURE 2

WEIGHT LOSS (%)

TIME (MIN)

150°C, one hour in 50% aqueous ethanol. At the higher temperatures weight loss approaches 50% for the case when both steps are performed.
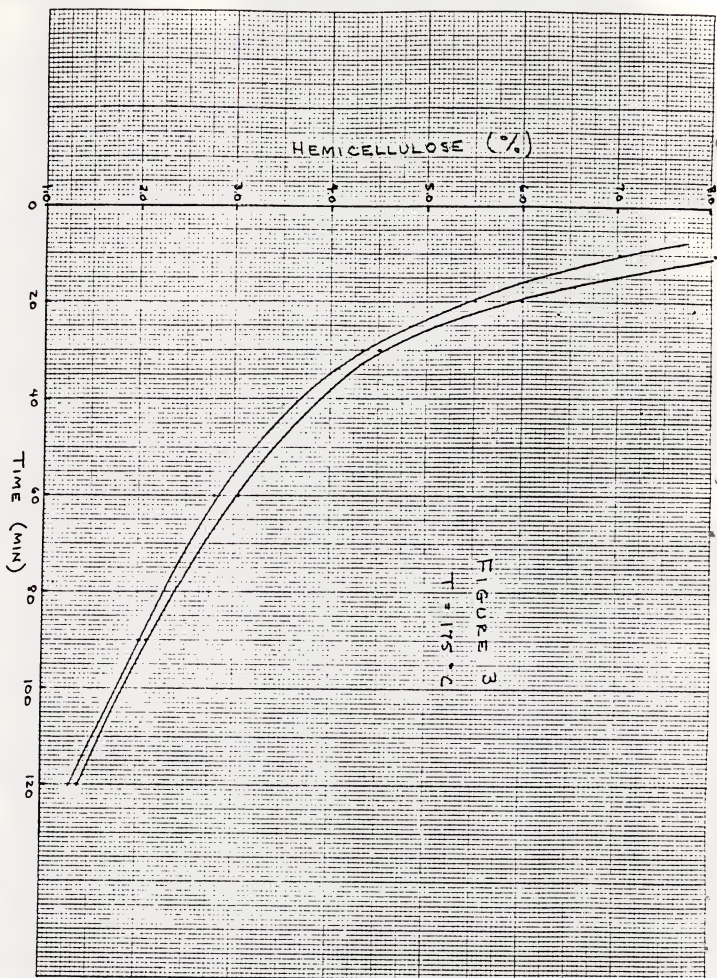
In Figure 1 the cross of the 195°C and 205°C lines is surely experimental error due to the limited data and lengthy intervals involved. The "true" spatial relationship of these lines can be inferred from the corresponding curves of Figure 2.

The high weight losses are somewhat surprising considering the starting wheat straw composition (extract-free, dry basis):

Ash	5.8%
Lignin	30.8%
Cellulose	46.7%
Hemicellulose	16.7%

Based upon the general literature, one would expect most of the hemicellulose to be lost to the liquor phase, but obviously significant lignin and cellulose components are being removed in autohydrolysis. These losses complicate any envisioned commercial process, since a major separation of the liquid stream would be required. Perhaps the use of steam only (no liquid water) would change these losses, but that seems unlikely from a chemical standpoint.

Decline of hemicellulose at autohydrolysis temperatures of 175°C and 195°C is shown in Figures 3 and 4, respectively. As expected, hemicellulose falls rapidly as it is hydrolyzed and the pentose sugars dissolve in the autohydrolysis liquor. In each figure the upper line represents solid residue hemicellulose after autohydrolysis only, and the lower line is residue hemicellulose after both autohydrolysis and delignification operations. As expected, the two curves almost coincide at 195°C, since little further hemicellulose is removed at 150°C in the delignification. No hemicellulose curve is given for 205°C as removal is almost complete, even at the shorter exposure intervals. Also, experimental precision is poor for analyzing hemicellulose concentrations below 1.0%.

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Figure 3
T = 175°C

Figure 4. $T = 195°C$

Hemicellulose (%) vs. Time (min)

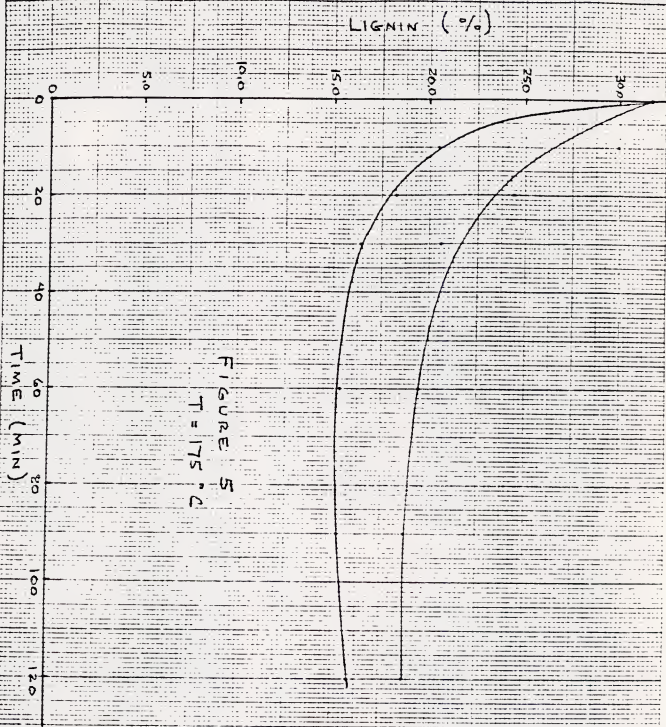22-116 · CROSS SECTION · 20 SQUARES TO INCH
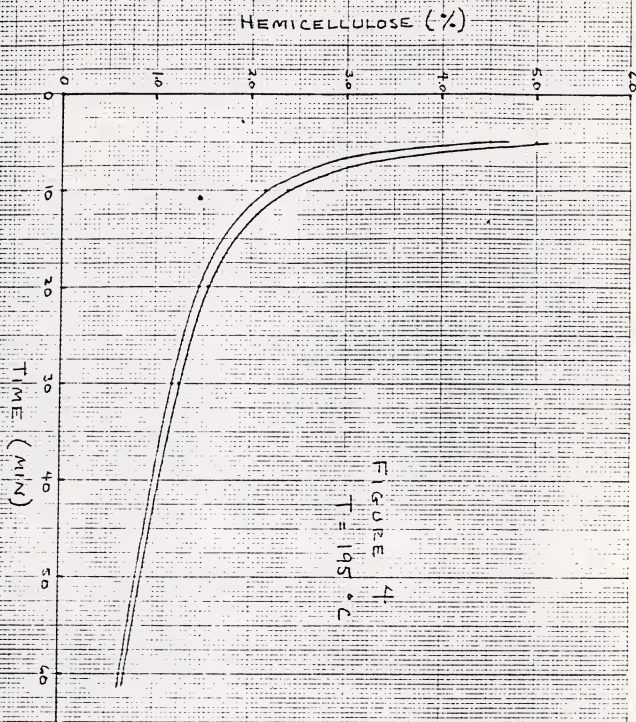
Several conclusions seem valid from the standpoint of optimizing autohydrolysis times and temperature based upon hemicellulose yield. At 175°C little is gained by autohydrolysis times longer than 90 minutes, and the same can be said for 20-30 minutes at 195°C. Although only an estimate, no more than 10 minutes should be required to give high hemicellulose yields at 205°C. Note also in Figures 1 and 2 that, at any of these tentative autohydrolysis exposure times, total weight loss is near the maximum for the respective temperatures.

Figures 5, 6 and 7 show residue lignin concentrations at 175°C, 195°C and 205°C corresponding to the hemicellulose results in Figures 3 and 4. Again, in each figure the upper curve gives lignin after autohydrolysis only and the lower curve after autohydrolysis and alcoholic delignification. For each temperature more than half of the original lignin is removed at certain intervals for the combined operations.

The results given in Figures 5, 6 and 7 are very interesting in several ways. It is seen that autohydrolysis alone gives significant lignin reduction at each temperature. This result is surprising, as one might not expect even fragmented lignin to dissolve so well in the aqueous autohydrolysis liquor. As mentioned above this is a problem from a scale-up standpoint since the hemicellulose stream would be contaminated with significant amounts of lignin, requiring a separation that may prove costly and difficult.

While the autohydrolysis-only curves go flat with time, distinct minima exist for residue lignin concentration at each temperature when the two process steps are combined. These minima are roughly 12.3% lignin for 10 minutes at 205°C, 13.5% lignin for 25 minutes at 195°C and 15.0% lignin for 70 minutes at 175°C. It seems reasonable to consider these conditions as optimal for each temperature since hemicellulose yield is near a maximum at the times involved.

The upward curvature exhibited by the lower curves at longer intervals may be due to repolymerization of lignin fragments into a form more intractable than the original lignin structure. Lora and Wayman (10) proposed this
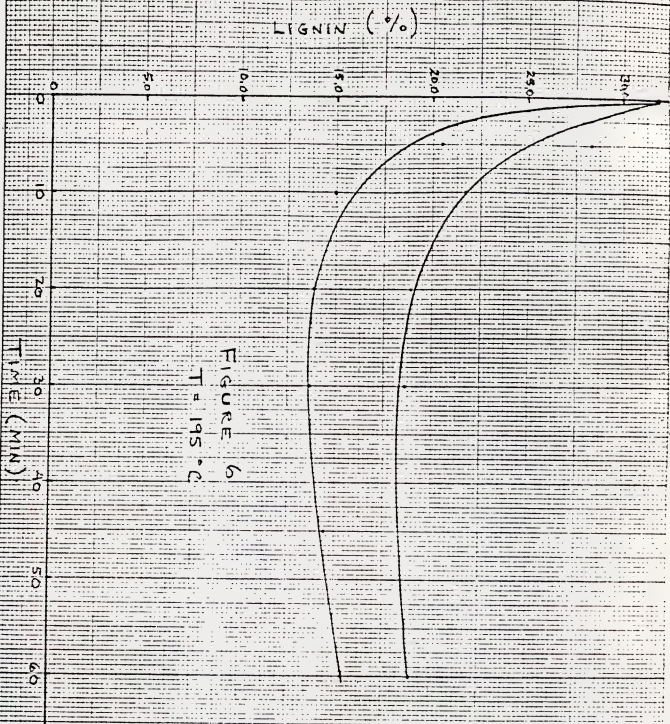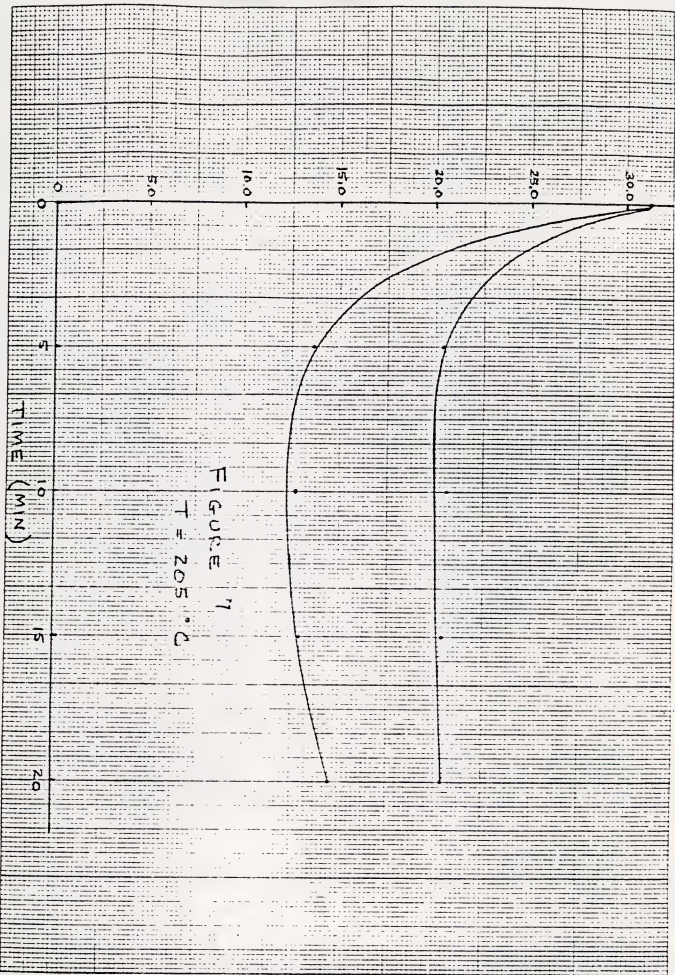
20

FIGURE 5
T = 175° C

LIGNIN (%)

Time (min.)

FIGURE 6
T = 195 °C

FIGURE 7
T = 205° C

22-116 · CROSS SECTION · 20 SQUARES TO INCH

mechanism upon observing similar behavior when extracting lignin with dioxane from autohydrolyzed poplar. Whatever the mechanism, extended autohydrolysis times beyond those required for high yields of hemicellulose are clearly nonproductive.

Although more than 70% of the original lignin is removed after alcoholic delignification of wheat straw autohydrolyzed at 205°C for 10 minutes, the effect of this lignin removal on the ultimate pulp cellulose hydrolysis by either acid or enzymes was unknown. Clearly the cellulose (alpha and beta) concentrations of residues from autohydrolysis alone and of those from the combined operations were increased as hemicellulose and lignin were removed. These results are given in Figures 8, 9 and 10 for 175°C, 195°C and 205°C, respectively. In these figures the lower curves are for autohydrolysis only and the upper curves represent the combined steps. The figures plot residue concentrations, not total cellulose. The latter quantity will never increase in the solids.

Figures 8, 9 and 10 show the response anticipated from earlier hemicellulose and lignin results. Cellulose increases as hemicellulose and lignin are removed, but declines somewhat at higher temperatures and longer autohydrolysis intervals as intractable lignin develops in autohydrolyzed and delignified pulps. Maxima in these curves occur at roughly the same autohydrolysis exposure times as lignin minima.

At this point in the research a reasonable overall optimum autohydrolysis set of conditions is ten minutes at 205°C. This gives essentially complete hemicellulose conversion and the lowest lignin concentration of any wheat straw pulp. Total weight loss is only a little higher than for the optimal conditions at 175°C and 195°C.

Acid Hydrolysis

The ultimate goal of this project is to produce biomass pulps of high cellulose content and in which the cellulose

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FIGURE 8
T = 175° C

FIGURE 9

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CELLULOSE (%)

FIGURE 10

T = 205 °C

TIME (MIN)
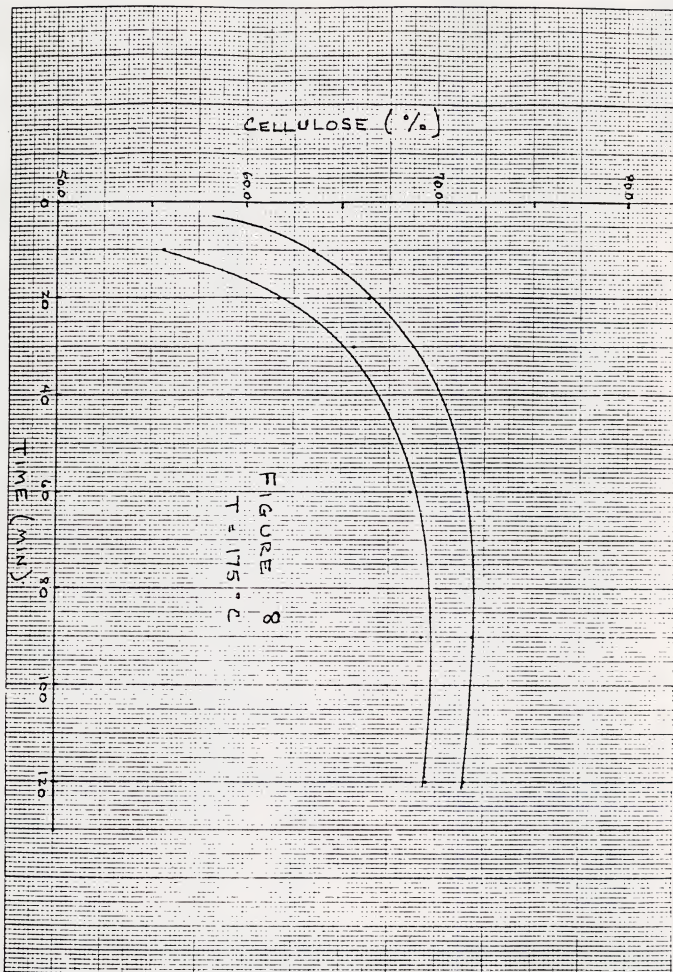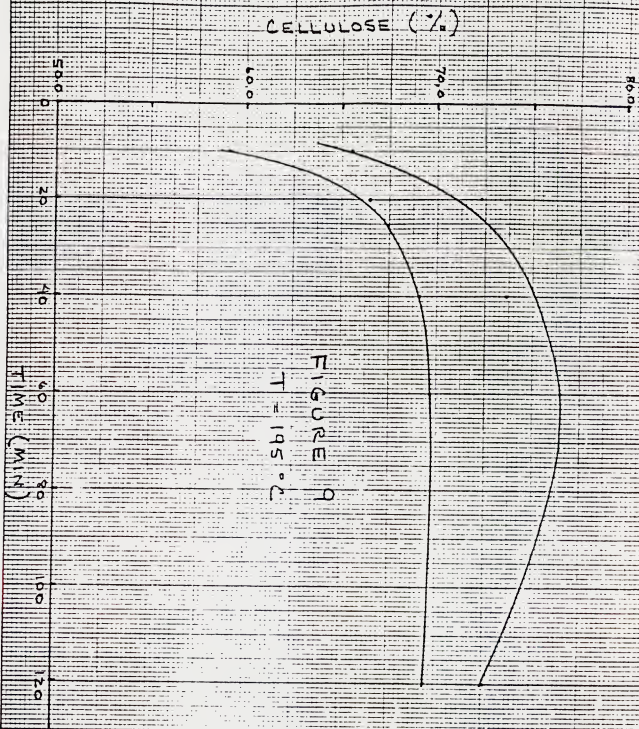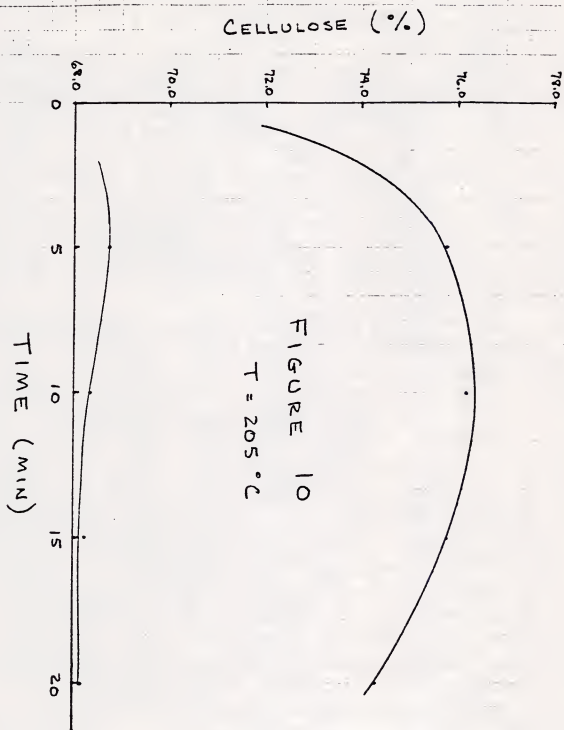
hydrolyzes readily to glucose for fermentation. It was hoped that the delignified pulps from autohydrolysis at 205°C for 10 minutes would achieve this goal. Any commercial process resulting from extension of the current project would then use continuous processing at roughly these conditions.

Today, pulp hydrolysis to glucose would be performed with enzymes which give high glucose yields and which don't require expensive process materials of construction as do acids. However, enzyme research is not within the limited scope of the current project. Therefore biomass pulps must be evaluated using acid hydrolysis in glassware. As reported earlier, a modified version of ASTM D-1106-56 was finally selected to rate cellulose pulps. The test result is a simple weight loss with the ease of cellulose hydrolysis figured to be proportional to the weight loss value.

The modified ASTM D-1106-56 test gave moderate weight losses on extract-free biomass starting materials on a dry basis:

Wheat Straw	35.8%
Barley Straw	32.8%
Lodgepole Sawdust	18.0%
Douglas Fir Sawdust	17.8%

Results are quite close within the two classes of materials, an encouraging result. It was also felt that the weight loss magnitudes, and hence test severity, were about right and probably would yield near complete cellulose weight loss for the optimal pulps. The lower weight losses shown above for the two woods are unexplained but surely have to do with varied lignocellulosic morphology among the materials. Analyses of all extract-free (gum/resin extraction) starting materials are given in Table 1 on a dry basis.

Table 2 gives analyses of extract-free materials after autohydrolysis (but not delignification) at autohydrolysis conditions found optimal for wheat straw (10 minutes at 205°C). Table 3 gives analyses of extract-free materials after both autohydrolysis at the optimal conditions for

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wheat straw and standard delignification. Weight loss as given in these tables is for the pretreatment steps (autohydrolysis and delignification) - not acid hydrolysis. Weight loss in Table 3 is total loss through both pretreatment steps. Clearly, Tables 2 and 3 give results for barley and the woods corresponding to those for wheat given in Figures 1, 2, 7 and 10 at 10 minutes autohydrolysis exposure.

A close look at Tables 2 and 3 reveals a number of facts. Weight losses are similar to the wheat values of 43.0% and 48.8% in Figures 1 and 2. However, this is where the similarities end. After autohydrolysis and autohydrolysis/delignification wheat straw hemicellulose falls to less than 1.0% while values in Tables 2 and 3 are much higher. An even greater discrepancy is seen in lignin results where only slight or no removal is achieved for the woods at the conditions found optimal for wheat straw. Barley shows similar behavior to the wheat straw lignin values of Figure 7. Cellulose values for all materials are in line with the hemicellulose and lignin results.

Thus, we surely do not have an optimal pretreatment procedure for lodgepole pine or Douglas fir as hemicellulose yields are only fair and the data show increases in lignin concentrations after pretreatment. The increases are probably not real, but reflections of the experimental precision in the lignin analyses. However, little or no lignin has been removed.

For barley the situation is better and we may be close to an optimal pretreatment. However, as with the woods, the hemicellulose yield in autohydrolysis is only fair. As noted above lignin results closely match those for wheat straw.

Acid hydrolysis of autohydrolyzed and delignified pulps using the test which gave encouraging results on starting materials gave very surprising results. As shown in Table 4, weight losses of pulps in acid hydrolysis were much lower than those given by the starting materials without pretreatment.

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Table 1

	Ash	Lignin	Hemicellulose	Cellulose
Wheat Straw	5.8%	30.8%	16.7%	46.7%
Barley Straw	4.3	27.5	18.0	50.3
Lodgepole Pine	0.2	26.0	22.6	51.2
Douglas Fir	0.1	26.6	21.6	51.7

Table 2

	Ash	Lignin	Hemicellulose	Cellulose	Weight Loss
Barley Straw	7.6%	16.6%	4.8%	71.9%	43.9%
Lodgepole Pine	0.3	28.1	7.5	64.0	34.8
Douglas Fir	0.1	29.5	3.4	67.0	35.2

Table 3

	Ash	Lignin	Hemicellulose	Cellulose	Weight Loss
Barley Straw	7.9%	12.5%	3.9%	75.6%	46.7%
Lodgepole Pine	0.3	29.6	3.8	66.3	36.1
Douglas Fir	0.1	27.6	3.8	68.4	37.1

	Table 4	
	Weight Loss (A)	Weight Loss (A,D)
Wheat Straw	7.7%	4.1%
Barley Straw	6.4%	4.6%
Lodgepole Pine	3.8%	3.2%
Douglas Fir	3.1%	2.6%

The first weight loss column (A) is for pulp having been through autohydrolysis only (Table 2), and the second column (A,D) is weight loss for pulp having been through both autohydrolysis and alcoholic delignification (Table 3).

Little acid hydrolysis of the wood species might be expected since the pretreatment step(s) barely influenced lignin concentrations (if at all), and much of the easily hydrolyzed hemicellulose is now missing after pretreatment. However, the very low acid hydrolysis values for the pretreated straws, which have lignin concentrations of less than 50% of starting materials, is unexpected.

Reasons for the low acid hydrolysis results given in Table 4 for wheat and barley straw are not certain, but several possibilities exist. Although treated pulp (both steps) lignin concentrations are only 41% and 45% of starting material values for wheat straw and barley straw, respectively, this order of lignin removal may not be sufficient to speed cellulose hydrolysis appreciably. The remaining intractable lignin may be that fraction most integrally "locked-up" with the cellulose. Thus, only the outer layers of the protective lignin sheath may be removed, and this may not be enough to allow improved access of hydrolytic species to the cellulose.

Another factor may also be involved, and that is high cellulose crystalline order even after pretreatment. After autohydrolysis and delignification, the cellulose concentrations of wheat and barley pulps were 75.8% and 75.6%, respectively. These pulps were examined by TAPPI method T203 os-74 for crystalline (alpha) and amorphous (beta) cellulose concentrations. For barley only 8.8% of the cellulose was amorphous, and essentially no beta cellulose was found in the wheat straw pulp. The test for

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beta cellulose is very subjective, and results are probably not very precise, but it is clear that the great majority of cellulose in the "optimal" pulps was crystalline as in the starting materials.

Autohydrolysis and delignification steps as performed in this study did not significantly disrupt the cellulose crystalline order as anticipated. Thus, physical access of hydrolytic species to reactive sites in cellulose molecules was apparently not improved and hydrolysis rate was not enhanced. Perhaps more severe temperatures in auto-hydrolysis and/or delignification are required or a stronger delignification solvent. These are clearly matters for further research.

Copies of the raw data for trials reported in this Results and Discussion section are given in the Appendix.

Recommendations for further research as given in the following section are currently being pursued under a one-year research grant from the Montana State University Engineering Experiment Station, Grant #16231013. The DNRC project reported herein was the "seed" basis for the MSU continuation grant. Results of continued research, especially results of potential commercial significance in Montana, will be made available to DNRC. An article to be published in the open literature is being prepared based upon this project, and DNRC will be cited as the sponsor (copies sent to DNRC).

Conclusions

Major conclusions reached in the research thus far are given below.

1) Optimal autohydrolysis and delignification as determined in this study reduce lignin concentrations of wheat and barley straw by nearly 60% and give xylose yields from hemicellulose of near 100% for wheat and 98% for barley. Significant losses of both hemicellulose and lignin in the autohydrolysis operation pose a separation problem for commercial operations.

2) Autohydrolysis and delignification conditions found optimal for wheat straw give only fair yields of xylose from lodgepole pine and Douglas fir and remove essentially no lignin from these starting materials.

3) Experimental apparatus as developed in this study works very well for the research purposes intended. A novel autoclave configuration probably yields better batch data than is generally being reported in the open literature.

4) Standard biomass analytical methods given by ASTM and TAPPI work reasonably well for the four substrates (wheat straw, barley straw, lodgepole pine and Douglas fir) employed in this study.

5) Wheat and barley straw pulps produced at "optimal" pretreatment conditions failed to exhibit enhanced rates of cellulose acid hydrolysis in a test developed in this study. It is presumed that enzymatic hydrolysis of these pulps would also be slow. Wood pulps also show low rates of acid hydrolysis as expected from their high lignin contents.

Recommendations for Further Research

The research thus far shows good promise overall. Experimental apparatus and techniques developed in this

project work well and are state-of-the-art for research conducted batchwise. Biomass analytical techniques in general use work reasonably well for Montana-specific substrates. Pretreatments studied to this point give overall good sugar yields from hemicellulose and moderate reductions of substrate lignin for straw. Recommendations for further research to enhance yields of glucose from pretreated pulps are given below.

1) Autohydrolysis temperatures above $205^{O}C$ coupled with delignification temperatures above $150^{O}C$ at "optimal" exposure times should be investigated. One-step combined autohydrolysis/delignification at higher temperatures seems promising.

2) Delignification solvent type should be varied in future research. Specifically, higher ethanol concentrations above 50% should be investigated as well as other organic solvents (i.e. n-butanol).

3) Physical destruction of cellulose crystallinity coupled with autohydrolysis/delignification should be studied. Such techniques as steam explosion and micro attrition may have merit to this end.

4) A detailed study of a single wood species (as with wheat straw) seems desirable. Clearly, whatever "optimal" conditions are found for this wood will differ appreciably from those for wheat straw.

APPENDIX

Budget

The overall RAE-83-1031 project budget is unchanged from the total in the original proposal of $23,162.00. Several budget category revisions were requested and approved by DNRC, but the grand total project budget is unaffected.

Timeline

The termination date of this contract was extended three months from September 30, 1984 to December 30, 1984. Several reasons forced the extension. Autoclave leaks and attendant experimental and safety concerns delayed early trials. Near the end of experimentation, major problems were encountered in developing an adequate acid hydrolysis test for pretreated pulps. Nearly two months were expended developing a test that originally was considered only a minor obstacle.

"AUTOHYDROLYSIS AND ALCOHOLIC
DELIGNIFICATION OF RENEWABLE CELLULOSIC BIOMASS"

Milestone Report #1

Reactor Description

An Autoclave Engineers Model ZC100 pressure autoclave reactor has been purchased for autohydrolysis and delignification experiments. The one-liter autoclave is frame mounted and constructed of 316 stainless steel throughout. The unit is pressure tested to 3000 psig and rated at 2000 psig.

Agitation is achieved via a turbine impeller magnetically coupled to an air motor. No shaft seal is involved. Air to the drive motor is regulated to constant pressure and oil mist lubricated. Agitator shaft rpm is infinitely variable between 0 and 1700 rpm and picked-up and displayed on an integral tachometer.

Heat is supplied to the autoclave by a ceramic insulated electrical band heater rated at 1000 watts. An on-off temperature controller maintains temperatures within $\pm 2^{\circ}C$. System temperature is monitored by a type J-(iron-constantan) thermocouple fitted in a stainless thermowell.

A set of eight 35 mm slides showing the Model ZC0100 reactor in a temporary installation is attached.

Reactor Shakedown Testing

A group of tests were conducted to confirm that the purchased autoclave will adequately perform all anticipated experiments.

The air-driven agitator has been tested briefly at 2000 rpm and for periods of up to four hours at 750 rpm with no problems encountered. This latter figure is probably a maximum for most anticipated experiments. The system's air regulator adequately throttles down our house air pressure fluctuations such that agitation speed is controllable within $\pm 5\%$. After calibration, the unit's oil mist motor lubrication mechanism performs well.

As delivered, the autoclave leaked in tests with a water charge at 150 psig. A damaged o-ring on a safety device intended

to safeguard against overpressure was found. After replacement, the autoclave was pressure tested to 350 psig with no leakage of the water charge. Tests to 500 psig with an ethanol charge are underway. Maximum anticipated pressure in this work is 350 psig.

Rapid heating and cooling of autohydrolysis mixtures is critical in this study. To test heat-up and cool-down rates in the autoclave, 500 ml of water at 20°C was heated at a controller set point of 250°C and then allowed to cool after reaching 210°C. Stirring rate was maintained at 750 rpm. A plot of the data obtained is attached.

Approximately 30 minutes is needed to raise the water temperature from 20° to 210°C. In autohydrolysis, appreciable reaction rates exist above 170°C. This unit requires about eight minutes to heat from 170°C to 210°C and about 30 minutes to cool from 210°C to 170°C with no venting.

The measured rate of heating is probably adequate for investigating autohydrolysis temperatures up to 190 or 195°C. For higher temperatures, autohydrolysis times of 10 minutes or less are required, and thus some auxiliary heating is needed. We are evaluating use of an existing high pressure bomb as a mini steam generator for this purpose. Rapid cool-down from all temperatures above 170°C will be achieved by venting the autoclave to atmosphere through cold traps to condense volatiles.

Overall, the Autoclave Engineers Model ZC0100 appears highly satisfactory for anticipated autohydrolysis and delignification experiments. Autoclave trials during the next few weeks will involve definition of experimental techniques and procedures.

Substrate Materials

Two bales each of Pondera spring wheat and Clark's barley have been obtained from the Larry Van Dyke Ranch in Amsterdam, Montana. Primary experimental emphasis will be on the wheat straw.

We will obtain stocks of douglas fir and lodge pole pine (probably sawdust) within several weeks.

Analytical

Most glassware, instrumentaion and reagent chemicals for analyses of starting materials and products are on hand. The major techniques (ASTM and TAPPI standard methods) have been scouted with no major problems encountered. The following tests have been conducted with 35 - 60 mesh ground wheat straw:

(1)	ash determination	ASTM D-1102
(2)	gum/resin extraction	ASTM D-1105
(3)	acid hydrolysis determination of lignin	ASTM D-1106
(4)	cellulose determination by chlorine delignification	TAPPI T9M
(5)	cellulose component splitting	TAPPI T203 OS-74

A full report on these analytical methods and auxiliary procedures along with data for wheat straw will be given in the Milestone #2 report.
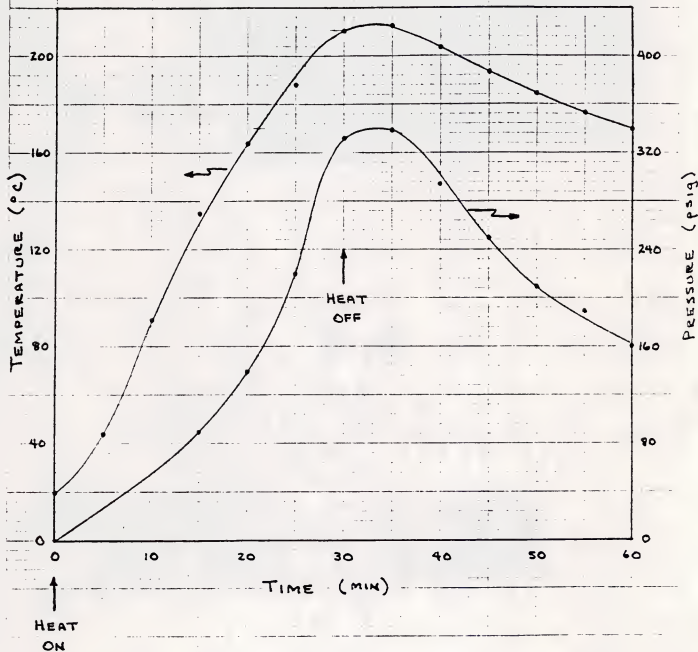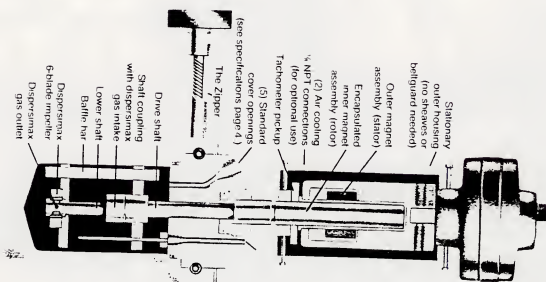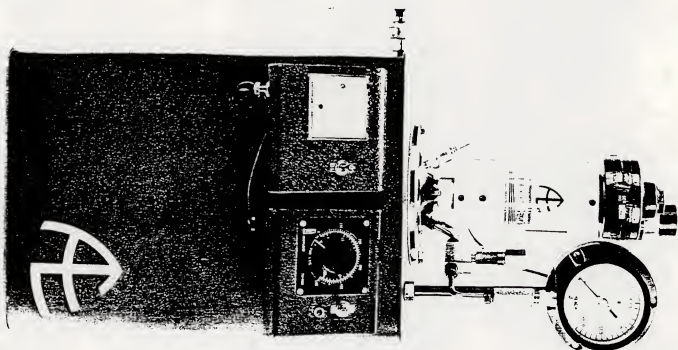
A-5

Dispersamax gas outlet

Dispersamax 6-blade impeller

Baffle bar

Lower shaft

Shaft coupling with dispersamax gas intake

Drive shaft

The Zipper

(see specifications page 4.)

(5) Standard cover openings

Tachometer pickup

¼ NPT connections (for optional use)

(2) Air cooling (for optional use)

Encapsulated inner magnet assembly (rotor)

Outer magnet assembly (stator)

Stationary outer housing (no sheaves or bellguard needed)

Milestone Report #2

* Analytical Status

A sequence of laboratory analytical techniques has been confirmed by
demonstration to accurately yield the composition of a lignocellulosic sub-
strate in terms of the following component breakdown:

    (a) moisture

    (b) ash

    (c) ethanol/benzene extractables

    (d) lignin

    (e) combined cellulose and hemicellulose

The standard techniques used by wood chemists to differentiate among
crystalline cellulose, amorphous cellulose and hemicellulose have not worked
with wheat straw and may require modification.

A summary of individual techniques and data for Pondera Spring Wheat
straw follow. Sample calculations are given as an appendix.

* Moisture Determination

Wheat straw milled to 35-60 mesh was conditioned for 24 hours at 68°F and
25% relative humidity. Conditioned straw was then weighed into bone-dry,
uncovered crucibles and dried for 12 hours in a convective air oven at 105°C.
An average moisture content of 5.45% was determined by weight loss for five
straw samples. Moisture content range was 0.17% for these five samples.
Duration of drying is rather arbitrary as organic volatile material continues
to escape from straw samples over prolonged intervals.

* Ash Determination

Ash is determined by ASTM Standard Method 1102, developed for wood.
Straw samples dried as for moisture determination were weighed into bone-dry
crucibles and combusted in air in a convective oven at 600°C for 30 minutes.
An average dry basis ash content of 9.34% was determined by weight loss for
three straw samples. Ash content range was 0.26% for these samples.

* Extractables Determination

Gums, resins and tannins were extracted from straw samples by TAPPI Standard Method T12 OS-75. Milled straw samples were sequentially extracted with benzene/ethanol solvent and 95% ethanol solvent in a Soxhlet apparatus. These extractions were followed by a final boiling water wash step. Percent extractables is determined on a dry basis by weight loss. Wheat straw yielded 15.80% extractables in a last trial using our best techniques. This result is very sensitive to mechanical loss of milled straw during filtration and handling.

* Lignin Determination

Lignin content of wheat straw is determined by TAPPI Standard Method T9M for wood chips. Starting material is fully dried, extracted straw. Lignin is broken down with chlorine gas and washed from the substrate with dioxane, monoethanolamine, distilled water and finally, ethyl ether. Lignin content is determined by ultimate weight loss on a dry basis. With our best techniques, lignin was determined as 19.96%. Again, this result is very sensitive to mechanical straw loss during handling.

* Cellulose and Hemicellulose Determination

Results presented thus far on dry and absolute bases are:

	Dry	Absolute
Moisture	---	5.45%
Ash	9.34%	8.83
Extractables	15.80	14.94
Lignin	19.96	18.88
	45.10	48.10

On a dry basis, hemicellulose and cellulose should total approximately 55% by difference. This result is well within the range of reported values for other wheat and barley straws.

However, our use of TAPPI Standard Method T203 OS-74 to split total cellulosic material into cellulose and hemicellulose fractions has not yielded satisfactory results. The procedure, developed for wood, is complex (Appendix II) and utilizes colorimetric titrations with (in our experience) vague end-points for wheat straw substrate. We are working to find any subtle problems

with techniques or reagents, but may be forced to utilize potentiometric titration instead of color indicators. This would involve selection and purchase of appropriate titrimeter electrode(s) and some technique development. Work on this project aspect is proceeding at high priority, as auto-hydrolysis and delignification trials require cellulose/hemicellulose determination. Progress will be detailed in the Milestone #3 Report.

* Reactor Developments

As reported in Milestone #1, reactor heat-up and cool-down over the 170-210°C range required about nine and thirty minutes, respectively. In this prior work the only heater utilized was the reactor bomb resistance unit and cooling was by natural convection.

To investigate autohydrolysis times of ten minutes or less near 200°C, supplemental heating and a different cooling mechanism are required.

A second autoclave (Parr Model 4052) can be used as a high pressure steam generator to accelerate heating over the critical 185-210°C temperature range. By injecting live steam initially at 315°C (1500 psig) into the AE Model ZC0100 autohydrolysis autoclave, temperature can be raised from 185°C to 210°C in less than 28 seconds. This compares with over six minutes for the same temperature rise without supplemental heating.

An evaporative cooling technique, where autohydrolysis liquor at temperature is throttle-vented through cold traps to the atmosphere, radically speeds reactor cool-down. Roughly 45 seconds is required to cool from 200°C to 180°C by a throttled venting procedure with no loss of reactor liquid contents. This interval compares with nearly eleven minutes to accomplish the same reactor cooling convectively.

Sample Calculations

* Moisture

Dry Crucible (A)	17.5834 gm
Dry Crucible and Moist Straw (B)	18.8232 gm
Moist Straw (B - A)	1.2398 gm
Dry Crucible and Dry Straw (C)	18.7553 gm
Moisture (B - C)	0.0679 gm
% Moisture [(B - C)/(B - A)] x 100	5.32%

* Ash (Dry Basis)

Dry Crucible (A)	32.1400 gm
Dry Crucible and Dry Straw (B)	34.0052 gm
Dry Straw (B - A)	1.8652 gm
Dry Crucible and Dry Ash (C)	32.3135 gm
Dry Ash (C - A)	0.1735 gm
% Ash [(C - A)/(B - A)] x 100	9.30%

* Extractables (Dry Basis)

Dry Straw - tared (A)	28.930 gm
Extracted Dry Straw - tared (B)	24.360 gm
% Extractables [(A - B)/(A)] x 100	15.80%

* Lignin (Dry Basis)

Dry Extracted Straw - tared (A)	3.1795 gm
Dry Delignified Straw - tared (B)	2.4256 gm
Lignin (A - B)	0.7539 gm
Dry Unextracted Straw (C)	3.7761 gm
$\quad$ C = (A)/(1.0000 - 0.1580)	
% Lignin [(A - B)/(C)] x 100	19.93%

Literature Survey

A comprehensive literature survey of research dealing with pretreatments of straw and woody biomass for enhanced delignification and hydrolysis is complete. Availability of DIALOG computer database searching in MSU's Renne Library made a trip to a research library such as SERI in Colorado unnecessary.

Two primary databases were employed in this search. The first file searched was CRIS (Current Research Information System). CRIS is a USDA database for agriculturally related research projects currently underway. Coverage includes most research sponsored by USDA, state agricultural and forestry experiment stations, and other cooperating state institutions. This database is updated bi-weekly.

The second file searched was PAPERCHEM, a comprehensive database covering worldwide research literature on pulp and paper technology, the chemistry of cellulose, hemicellulose, carbohydrates and lignin, and a wide variety of related topics. Nearly 1,000 journals and proceedings are screened as well as the international patent literature. Finally, PAPERCHEM overviews books, dissertations, review articles, symposia and technical translations. This database is updated on a monthly basis.

From among hundreds of citations, Appendix I lists thirty recent publications dealing with pretreatments of lignocellulose

prior to hydrolysis. References are grouped by principal substrate investigated and method(s) of pretreatment. Several summary review articles, pilot plant projections, and miscellaneous alternative approaches are also included in the listing. Among the review articles, that by Blanch and Wilke (1983) is especially useful as a starting point.

Other Progress

The colorimetric end point problem with TAPPI Standard Method T203 OS-74 has been solved by potentiometric end point determination. An existing MSU titrimeter electrode is being successfully used in this test method that splits total cellulose into hemicellulose and cellulose fractions.

Another recent problem of rupture disc failure on our small steam generation bomb is resolved. A previously unexplained failure of a 6000 psi disc at less than 1500 psi was apparently due to age and thermal fatigue. New rupture discs solve the problem and eliminate an important safety concern with the work.

A. Studies using Wheat Straw as the Principal Substrate

Pretreatment Mode: Autohydrolysis

1) Lewis, R.A. "Sugar Production during Autohydrolysis of Wheat Straw", Proceedings from the Annual Biochemical Engineering Symposium (Fort Collins Colorado), 11(1981).

2) Lewis, R.A. "Combined Autohydrolysis-Organosolv Pretreatment of Lignocellulosic Materials", Proceedings of the Annual Biochemical Engineering Symposium (Ames, Iowa), 1(1980).

3) Linden, J.C., Murphy, V.G., Moreira, A.R. "Wheat Straw Autohydrolysis", Proceedings from the International Fermentation Symposium (London, Ontario), 41(1981).

4) Murphy, V.G., Linden, J.C., Moreira, A.R, and Lenz, T.G. "Development of Geothermally Assisted Process for Production of Liquid Fuels and Chemicals from Wheat Straw", United States Department of Energy Report DE-AS07-79ID12051, (1981).

Pretreatment Mode: Solvent Extraction

5) Avgerinos, G.C., Wang, D.I. "Selective Solvent Delignification for Fermentation Enhancement", Biotechnology and Bioengineering, 25(1), 67(1983).

Investigations of Two or More Pretreatment Modes

6) Detroy, R.W., Lindenfelser, L.A. "Saccharification of Wheat-Straw Cellulose by Enzymatic Hydrolysis following Fermentative and Chemical Pretreatment", Biotechnology and Bioengineering Symposium No. 10, 135(1980).

7) Fan, L.T., Lee, Y., Gharpuray, M.M. "Evaluation of Pretreatments for Enzymatic Conversion of Agricultural Residues", Biotechnology and Engineering Symposium No. 11, 29(1981).

8) Gharpuray, M.M., Lee, Y.H., Fan, L.T. "Pretreatment of Wheat Straw for Cellulose Hydrolysis", Proceedings from the Annual Biochemical Engineering symposium (Fort Collins, Colorado), 1(1981).

B. Studies Using Wood Residues as the Principal Substrate

Pretreatment Mode: Autohydrolysis

9) Chua, M.G. "Characterization of Autohydrolysis Aspen (Populus Tremuloides) Extracted and Residual Lignins", University of Toronto, Ph.D. thesis (1979).

10) Lora, J.H., Wayman, M. "Delignification of Hardwoods by Autohydrolysis and Extraction", Tappi 61(6): 47(1978).

11) Lora, J.H., Wayman, M. "Autohydrolysis of Aspen Milled Wood Lignin", Canadian Journal of Chemistry, 58(7): 669(1980).

12) Wayman, M. "Fundamental Principles and Their Practical Consequences in the Conversion of Biomass to Ethanol", Proceeding of the Royal Society of Canada's International Symposium on Ethanol from Biomass (Winnipeg), G27(1983).

13) Wayman, M. "Alcohol from Cellulosics: The Autohydrolysis-Extraction Process", Alcohol Fuels Technology's 4th International Symposium Proceedings, 79(1981).

14) Wayman, M., Chua, M.G. "Characterization of Autohydrolysis Aspen (Populus Tremuloides) Lignins: Residual Autohydrolysis Lignin", Canadian Journal of Chemistry, 57(19): 2612(1979).

15) Wayman, M., Lora, J.H., Gulbinas, E. "Material and Energy Balances in Production of Ethanol from Wood", ACS Symposium Series No. 90, 183(1979).

Pretreatment Mode: Solvent Extraction

16) April, G.C., Kamal, J.A. "Delignification with aqueous-organic solvents", Tappi, 62(5): 83(1979).

17) Phillips, J.A., Humphrey, A.E. "Process Technology for Biological Conversion of Lignocellulosic Materials to Fermentable Sugars and Alcohols", Proceeding from ACS Conference (Kansas City), Soltes, E.J. ed. (1983).

C. Review Articles

18) Allen, B.R., Cousin, M.J., Pierce, G.E. "Pretreatment Methods for Degradation of Lignin", National Science Foundation Grant No. PER 79-25833 Final Report, (1980).

19) Blanch, H.W., Wilke, C.R. "Sugars and Chemicals from Cellulose", Reviews in Chemical Engineering, 1(1): 71(1933).

20) Dhake, J.D., Khante, N.G. "Delignification of Agricultural Residues by Modified Soda Process", Indian Pulp Paper, 35(4): 9(1981).

21) Sarkanen, K.V., Tillman, D.A. "Progress in Biomass Conversion: Vol. 2", Academic Press, (1980).

D. Pilot Plant Projections

22) Becker, D.K., Blotkamp, P.J., Emert, G.H. "Pilot Scale Conversion of Cellulose to Ethanol", Fuels from Biomass and Wastes, Proceedings from ACS/AICHE meeting, (1981).

23) Clausen, E.C., Gaddy, J.L. "The Production of Ethanol from Biomass Lignocellulosic Materials by Acid Hydrolysis and Fermentation", University of Arkansas, (1980).

24) Taylor, J.D. "Continuous Autohydrolysis, a Key Step in the Economic Conversion of Forest and Crop Residues into Ethanol", Energy from Biomass Conference (Brighton), 330(1980).

25) Wilke, C.R. "Pilot Plant Studies of the Bioconversion of Cellulose and Production of Ethanol", U.S. Energy Research and Development Administration, Fuels from Biomass Program: Report of Progress, Lawrence Berkeley Laboratory, (1977).

E. Miscellaneous

26) Koukios, E.G., Valkanas, G.N. "Process for Chemical Separation of the Three Main Components of Lignocellulosic Biomass", I&EC Prod. Res., 21(2): 309(1982).

27) McCarty, P.L., Young, L. "Heat Treatment of Biomass for Increasing Biodegradability", 3rd Annual Biomass Energy Systems Conference Proceedings (Golden, Colorado), (1979).

28) Tanaka, M., Taniguchi, M. "Studies on the Reutilization of Cellulosic Resources: Effect of Chemical Treatment on Solubilization of Crystalline Cellulose and Cellulosic Wastes with Pellicularia Filamentosa Cellulase", Journal of Fermentation Technology, 57(3): 186(1979).

29) Taya, M., Honman, K. "Pretreatment of Cellulosic Materials for Digestion by Ruminococcus Albus", Journal of Chemical Engineering in Japan, 14(4): 330(1981).

"AUTOHYDROLYSIS AND ALCOHOLIC DELIGNIFICATION OF RENEWABLE
CELLULOSIC BIOMASS"

Milestone Report #4

* Particle Size Evaluation

Size reduction trials on Pondera spring wheat straw and
lodgepole pine substrates have yielded particulates ranging from
coarse chopped straw and sawdust to powders finer than 60 mesh.
Powders to 35/60 mesh were easily achieved using a standard Wiley
hammer mill. Finer powders at 60/80 mesh could not be readily
achieved due to mill overheating at extended milling times.

Autohydrolysis experiments originally planned to determine
required particle sizes for straw and wood in this study are not
required based upon microscopy studies of particles.
Photomicrographs at 12X (attached) show that at the easily
achieved 35/60 mesh both wheat straw and pine exhibit a platelike
particle shape. The thin dimension of these "plates" is roughly
20% of the width and an even smaller fraction of the length.
Thus, the thin dimension very roughly corresponds to a 175/300
mesh particle diameter for material fragments of a more spherical
nature.

Heat and mass transfer rates in autohydrolysis and extractive
delignification should be dictated by the thinnest particle
dimension. Since the shape of straw and pine particles yields
"diameters" equivalent to 175/300 mesh at the readily achieved
35/60 mesh, further grinding seems unnecessary. For reference,

250/300 mesh is nominally the particle size of fine grain flours. Particle size of 35/60 mesh will be used as standard in this research.

* Extraction Trials

Extraction trials using 50/50 benzene/ethanol solvent in a Soxhlet extractor yielded values ranging from 10-15% extractables for wheat straw and about 7% for lodgepole pine, each on an absolute basis (including moisture). Extracted material consists of gums, resins and tannins which are generally yellowish in color.

We have decided that all experiments in the current project must be conducted using extracted straw or wood. There are several reasons. First and foremost, TAPPI standard methods used to measure lignin, hemicellulose and cellulose dictate the use of extracted substate as starting material. Modification of these standard procedures would be difficult at best, and probably would yield results which could not be compared with those of other researchers. These procedures also involve colorimetric titrations which would probably be biased by the presence of the yellowish extractables.

Beyond the apparent analytical problems posed by the inclusion of extractables, there are other likely experimental problems. Gums, resins and tannins are chemically complex, and their chemical impact upon autohydrolysis could be significant. Researching and understanding this impact is beyond the scope of

our present investigation. Thus it again makes sense to utilize extracted substrates.

Any larger scale process utilizing this technology would probably not have a separate extraction operation, but in our fundamental work it seems a necessity.

* Other Results

We now have complete analyses on wheat straw and lodgepole pine and have evaluated the entire experimental sequence for each substrate, except for final pulp hydrolysis.   A complete description is forthcoming in Milestone #5.

Substrate analyses are (nominal):

	Wheat Straw	Lodgepole Pine
Moisture	5.5%	6.7%
Ash	8.8%	0.5%
Extractables	14.9%	7.0%
Lignin	18.9%	23.6%
Total Cellulose	51.9%	62.2%

These results are well in line with reports of other investigators.

"AUTOHYDROLYSIS AND ALCOHOLIC DELIGNIFICATION OF RENEWABLE CELLULOSIC BIOMASS"

Milestone Report #5

This milestone report details the experimental sequence of autohydrolysis, alcoholic delignification and final pulp hydrolysis. Key process variables are discussed, and results for wheat straw processing are given.

* Autohydrolysis

All autohydrolysis trials on wheat straw are complete. Temperatures of 175°C, 195°C and 205°C were investigated with contact times ranging from 5 minutes to 2 hours. Lignin content of solid residues declined markedly as autohydrolysis temperature increased, and at constant temperature lignin declined with increasing autohydrolysis time.

Loss of lignin into the autohydrolysis liquor was unexpectedly high in all trials. The starting autohydrolysis medium was 600 ml of distilled water into which about eight grams of ethanol/benzene-extracted wheat straw was immersed. Literature results on autohydrolysis of wood indicate that only hemicellulose is removed to a significant extent, but at 205°C and only 5 minutes of autohydrolysis contact more than 30% of the lignin was removed along with essentially all of the hemicellulose.

Dilution (water/straw ratio) was restricted to 75:1 on a mass basis due to design of the reactor agitator. Straw substrate was held in small wire baskets attached to the agitator shaft with the need to fill all baskets for stable agitation. A water charge of 600 ml was needed to cover the baskets with the agitator running. Since this quantity of water was already in excess of the amount envisioned in a scale-up of autohydrolysis, larger quantities up to the

1000 ml reactor capacity were not investigated.

Agitation was maintained at 200 rpm throughout autohydrolysis. The wire gauze that retained straw particles in the agitator baskets was 200 mesh. This fine mesh caused the baskets to act as "paddles" with very little liquor flow-through. Thus, it was felt that agitator speed would not appreciably influence system mass transfer. Stability of the agitator air motor was also a problem in the study. Stable agitator speeds could only be achieved at several discrete levels, with 200 rpm one of these levels.

Autohydrolysis vessel heat-up and cool-down times were minimized such that shorter contact times could be utilized without major impact of transient system behavior. Live steam injection and resistence jacket heating were combined to yield heat-up intervals (from 150°C to 170°-205°C) of less than 90 seconds. Cool-down was accomplished by venting to atmosphere through cold traps, with times to reach 150°C typically less than 90 seconds.

Sample quantitative results for 195°C/30 minutes autohydrolsis of wheat straw are given in Table 1. Basis for the results is ethanol/benzene-extracted, moisture-free straw.

* Alcoholic Delignification

Further lignin removal beyond that achieved in autohydrolysis was achieved in alcoholic delignification. In this operation autohydrolyzed straw is contacted with 50% aqueous ethanol at 150°C for 60 minutes. A fixed temperature of 150°C was selected for delignification as a compromise. This temperature should be maximized from the standpoint of lignin solubility, but other research on wood indicated autohydrolytic activity and lignin repolymerization at temperatures as low as 170°C. Thus, it was decided to go up to 150°C, but not to exceed it.

Delignification time was fixed at 60 minutes. To maintain aqueous alcohol composition at 50% by weight, steam injection to speed heating was not employed and heat-up times of 12-15 minutes were needed. To minimize the impact of this transient interval the fixed, relatively long delignification time of 60 minutes was utilized.

Agitation during alcoholic delignification was fixed at 200 rpm for the same reasons outlined above for autohydrolysis agitation.

All autohydrolysis residues at 175°, 190° and 205°C have been alcoholically delignified at the above standard conditions. Analytical results for material autohydrolyzed at 195°C for 30 minutes and then delignified is given in Table 1. Lignin reduction is seen by also comparing the last two columns.

* Cellulose Pulp Hydrolysis

Development of a satisfactory acid hydrolysis test for autohydrolyzed or autohydrolyzed/delignified residues of wheat straw is essentially complete after numerous trial-and-error attempts. First efforts to develop a test focused upon the use of sulfuric acid at strengths of 3 - 18 normality and temperatures of 40 - 80°C. In all cases, any combination of acid strength and temperature that resulted in greater than 50% weight loss of pretreated pulps (in less than two hours) resulted in excessive charring of residue materials. Conditions that gave little charring would not yield adequate weight loss for rating pulps and hence pretreatment schemes. High temperatures for very short contact times are indicated for any envisioned commercial acid hydrolysis process, but such procedures could not be employed in the present study.

In this work a compromise hydrolysis consisting of two steps will be employed to rate pretreated pulps. First, straw pulps are hydrolyzed with 16 N sulfuric acid for 60

minutes at 28°C. Following this step, pulp residue is further hydrolyzed and acid washed with 3 N sulfuric for 120 minutes at 80°C. This latter step does little hydrolysis, but washes product glucose out of the pulp residues. The ratio of acid to starting pulp in the concentrated step is 100:1, and dilution is used to bring the acid concentration to 3 N. The hydrolysis sequence described is a milder version of ASTM Standard Method D-1106 used to determine lignin by complete cellulose hydrolysis.

Results of hydrolyses of pretreated pulps will be detailed in the final project report. Initial hydrolysis results on wheat straw pulps indicate a strong influence of cellulose morphology. It appears that total weight loss of cellulose through autohydrolysis, alcoholic delignification and acid hydrolysis is limited to 40-50% at any combination of treatment conditions. Such results may be peculiar to wheat straw, and, if so, experiments now underway with barley straw, lodgepole pine and Douglas fir will show major differences.

Final experiments on the other three substances are in progress at conditions found optimal for wheat straw. Results will be detailed in the combined Milestone #6/Final Report.

Table 1

Results of Autohydrolysis ($195°C$ m 30 minutes)
and Alcoholic Delignification

	Substrate	Autohydrolysis Residue	Delignification Residue
Lignin	30.8 %	18.8 %	13.8 %
γ-Cellulose (Hemicellulose)	16.7 %	1.2 %	1.3 %
α,β Cellulose	46.7 %	69.7 %	73.9 %
Ash	5.9 %	10.3 %	11.0 %